

## Symposium: Probing Ion Channel Structure/Function Using Novel Tools

### 889-Symp

#### Conformational Changes in Voltage-Sensing Domains: Concerted Simulation and Scattering Studies

Douglas J. Tobias.

Chemistry, University California, Irvine, Irvine, CA, USA.

Electrical signals in excitable tissues such as nerves and muscles are generated by the synchronized opening and closing of ion channels in cell membranes, which generate transient transmembrane gradients in the concentrations of sodium and potassium ions. These channels open and close in response to local changes in transmembrane potential as the signal propagates, and it is well established that their voltage sensitivity is conferred by charged structural elements, referred to as voltage-sensing domains. The details of how conformational changes in the voltage-sensing domains lead to opening/closing of the ion-conducting pores of the channels are still being worked out. Crystal structures of the open states of voltage-gated potassium channels are available, but the structure of the closed/resting state has not been determined to high resolution experimentally. In this talk I will report our efforts to generate a model of the resting state of the archaean KvAP potassium channel based on atomistic molecular dynamics (MD) simulations in explicit membrane environments, with restraints derived from experimental functional data. I will also present results from simulation studies of an isolated voltage-sensing domain in a hydrated membrane, which include validation by neutron diffraction measurements, and direct observations of elementary gating charge displacement events during a 30-microsecond MD simulation under applied transmembrane potential. Finally, I will discuss prospects for and present preliminary results on using x-ray and neutron interferometry measurements, carried out by our collaborators, to validate and refine simulation-based models of the resting state and the voltage-sensing mechanism.

### 890-Symp

#### Tricking Out the Toolbox: Use of Genetic Code Expansion for the Study of Ion Channels

Chris Ahern.

Molecular Physiology and Biophysics, University of Iowa, Iowa City, IA, USA.

The application of nonsense suppression strategies to membrane proteins has great potential for the study and manipulation of ion channels and receptors. The technique relies on the incorporation of a stop codon that is subsequently 'suppressed' by an orthogonal misacylated tRNA. Ion channels in particular are especially amenable to these approaches due to the built-in signal gain from the high conductance of recorded ions. On one end of the scale of possibility, 'small mutagenesis' provides the means for single atom replacement within an amino acid or sub-atomic redistribution of pi electrons of aromatic side-chains. Such approaches provide an atomic resolution functional correlates to match high-resolution structural data, and have been key in decoding the interaction energetics of ligands, therapeutics, backbone contributions, salt-bridges and H-bonds in gating of voltage- and ligand-gated channels and receptors. More unconventional side-chains, such as light activated cross-linkers, hold promise for their ability to characterize transient noncovalent protein contacts. Lastly, encoded amino acid fluorophores, while highly valued, have been technically resistant to ion channel application and largely remain on the experimental fringe. Data will be presented on the use of genetic code expansion as a tool to reveal the basis for inactivation mechanisms employed by voltage-gated sodium and potassium channels.

### 891-Symp

#### Small Molecule Modulation of Voltage-Gated Ion Channels

Heike Wulff<sup>1</sup>, Vladimir Yarov-Yarovoy<sup>2</sup>.

<sup>1</sup>Pharmacology, University of California, Davis, Davis, CA, USA,

<sup>2</sup>Physiology and Membrane Biology, University of California, Davis, Davis, CA, USA.

Pharmacological modulation of voltage-gated-like ion channels offers tremendous opportunities for the discovery of new drugs for neurological, cardiovascular and metabolic disorders, as well as for immunosuppression and cancer. However, in contrast to other therapeutic targets such as protein kinases, voltage-gated ion channels have not been popular with medicinal chemists in the pharmaceutical industry. One reason for this unpopularity is the absence of co-crystals of medically relevant channels with drug molecules bound, a prerequisite for structure based drug design. To address this challenge, molecular modeling, which recently made significant advances due to improved computational methods, and the availability of more high-resolution ion channel structures, is increasingly being used to interpret the results of mutational and

electrophysiological experiments in structural terms. Using the Kv1.3 blocker PAP-1 and several KCa2/3 channel modulators such as TRAM-34, NS8593, and nifedipine as examples we here demonstrate that small molecule drugs can "snuggle" into every available nook and cranny of voltage-gated ion channels: the central cavity, the side-pockets between the pore-domain and voltage-sensor, the S5-S6 interface or the lateral fenestrations. We will further discuss the recent design of the KCa3.1 selective positive gating modulator SKA-121 (5-methylnaphtho[2,1-d]oxazol-2-amine), a compound that binds at the interface of the calmodulin N-lobe and the calmodulin binding domain of KCa3.1. Overall these examples illustrate that voltage-gated ion channels are highly accessible to small molecule drugs and that virtually any domain of these large, multi-subunit proteins can be targeted for the design of channel subtype-specific drugs, a major challenge remaining in the ion channel field. Supported by RO1 GM076063 (to H.W.) and UC Davis startup (to V.Y.).

### 892-Symp

#### Calcium Channel Engineering

Henry M. Colecraft.

Physiology and Cellular Biophysics, Columbia University, New York, NY, USA.

High-voltage-activated calcium (Ca<sub>v</sub>) channels convert electrical signals into Ca<sup>2+</sup> ion influx to trigger biological responses in excitable cells. Ca<sub>v</sub> channel dysfunction underlies many cardiovascular and neurological diseases, from cardiac arrhythmias to autism. Modulation of Ca<sub>v</sub> channels by intracellular signaling proteins, Ca<sup>2+</sup> ions and various small molecules is a powerful method to regulate physiology and is therapeutic for myriad disease conditions. I will discuss Ca<sub>v</sub> channel engineering approaches that we have used to gain insights into the structure-function mechanisms of these vital proteins, to probe their physiological roles, and to develop novel genetically-encoded molecules that modulate their activity.

## Platform: Calcium Signaling

### 893-Plat

#### Decreased Polycystin 2 Expression Alters Calcium-Contraction Coupling and Changes Beta-Adrenergic Signaling Pathways

Ivana Y. Kuo<sup>1</sup>, Andrea T. Kwaczala<sup>2</sup>, Lily Nguyen<sup>3</sup>, Stuart G. Campbell<sup>2</sup>, Barbara E. Ehrlich<sup>1</sup>.

<sup>1</sup>Pharmacology, Yale University, New Haven, CT, USA, <sup>2</sup>Biomedical Engineering, Yale University, New Haven, CT, USA, <sup>3</sup>Cardiology, Yale University, New Haven, CT, USA.

Cardiac disorders are the main cause of mortality in autosomal dominant polycystic kidney disease (ADPKD). However, how mutated polycystins predispose ADPKD patients to cardiac pathologies before development of renal dysfunction is unknown. We investigate the effect of decreased polycystin 2 (PC2) levels, a calcium channel that interacts with the ryanodine receptor, on myocardial function. We hypothesize that heterozygous PC2 mice (Pkd2+/-) undergo cardiac remodeling due to changes in calcium handling, separate to renal complications. We found that Pkd2+/- cardiomyocytes have altered calcium handling, independent of desensitized calcium-contraction coupling. Paradoxically, in Pkd2+/- mice, PKA phosphorylation of phospholamban was decreased, whereas PKA phosphorylation of troponin I was increased, explaining the decoupling between calcium signaling and contractility. In silico modeling supported this relationship. Echocardiography measurements showed that Pkd2+/- mice have increased left ventricular ejection fraction after stimulation with isoproterenol (ISO), a  $\beta$ -adrenergic receptor ( $\beta$ AR) agonist. Blockers of  $\beta$ AR-1 and 2 inhibited the ISO response in Pkd2+/- mice, suggesting that the dephosphorylated state of phospholamban is primarily by  $\beta$ AR-2 signaling. Importantly, the Pkd2+/- mice were normotensive and had no evidence of renal cysts. Our results showed that decreased PC2 levels shifted the  $\beta$ AR pathway balance and changed expression of calcium handling proteins, which resulted in altered cardiac contractility. We propose that PC2 levels in the heart directly contribute to cardiac remodeling in ADPKD patients in the absence of renal dysfunction.

### 894-Plat

#### In vivo Reconstitution of the Mitochondrial Uniporter

Erika Kovacs-Bogdan<sup>1</sup>, Yasemin Sancak<sup>1</sup>, Kimberli J. Kamer<sup>1</sup>, Molly Plovani<sup>1</sup>, Ashwini Jambhekar<sup>1</sup>, Robert J. Huber<sup>2</sup>, Michael A. Myre<sup>2</sup>, Michael D. Blower<sup>1</sup>, Vamsi K. Mootha<sup>1</sup>.

<sup>1</sup>Molecular Biology, Massachusetts General Hospital, Boston, MA, USA,

<sup>2</sup>Department of Neurology, Massachusetts General Hospital, Boston, MA, USA.

The mitochondrial uniporter is a highly selective calcium channel present broadly in eukaryotes, but absent in *Saccharomyces cerevisiae*. Therefore,